

Supplementary Material

A mutated Nme1Cas9 is a functional alternative RNase to both LwaCas13a and RfxCas13d in the yeast *S. cerevisiae*

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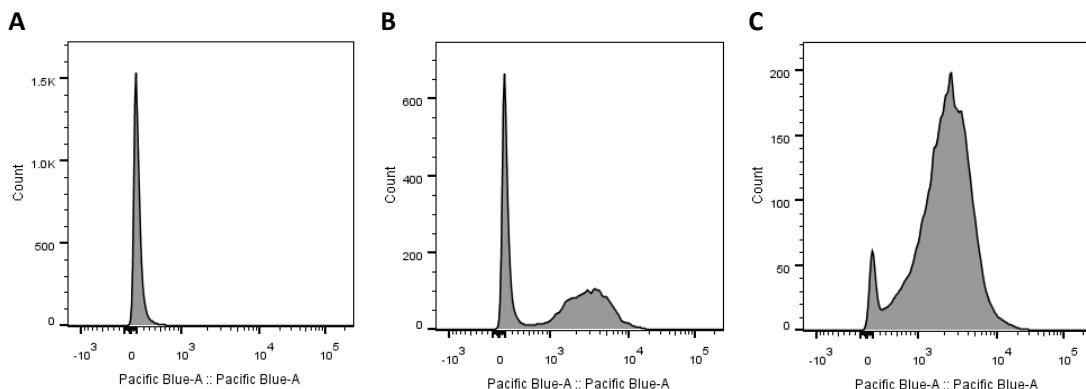


Figure S1. Fluorescence-Cell count histogram of FACS experiments. (A) Full gene editing; (B) partial gene editing with relatively low fluorescence; (C) partial gene editing with relatively high fluorescence.

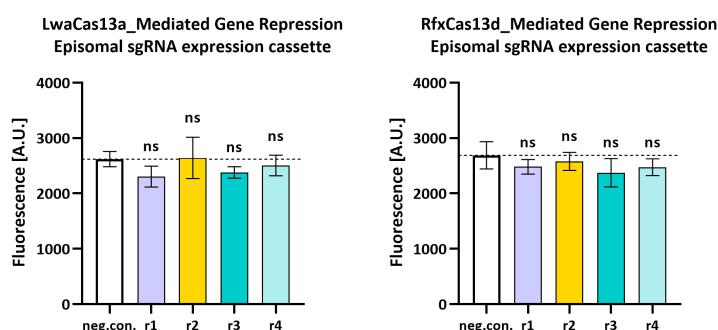


Figure S2. Fluorescence level of LwaCas13a/RfxCas13d-mediated gene repression when the sgRNA expression cassette was inserted into an episomal shuttle vector. “neg. con.” represents the negative control (no sgRNA was expressed). ri, i=1,...,4 stands for sgRNAr*i*. The black dashed line marks the fluorescence level of negative control. “ns” indicates no significant difference between the corresponding test strains and the negative control (two-tailed Welch’s t-test). Each fluorescence level represents the mean value from three independent measurements at the FACS machine. Error bars are the standard deviation of the mean.

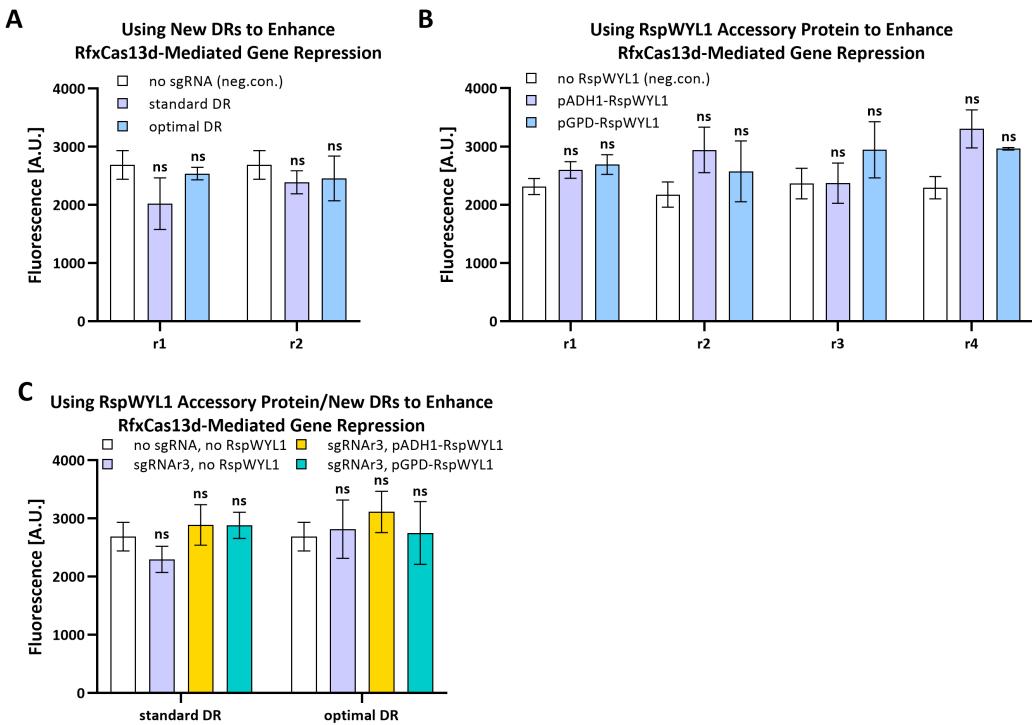


Figure S3. Fluorescence level of RfxCas13d-mediated gene repression. (A) New DRs replaced the one initially used. (B) RspWYL1 is added to the original circuit and expressed under either pGPD (a very strong promoter) or pADH1 (a moderately strong promoter). (C) The combination of new DRs and RspWYL1. ri, i=1,...,4, stands for sgRNAr*i*. “ns” indicates no significant difference between the corresponding test strains and the negative control (two-tailed Welch’s t-test). Each fluorescence level represents the mean value from three independent measurements at the FACS machine. Error bars are the standard deviation of the mean.

Table S1. Plasmids constructed in this work.

NAME	CONTENT
pMM1309	pRSII404-pTEF2-yEBFP2-CYC1t_atc
pMM1379	pRSII404-DEG1t_pCYC1noTATA-yEBFP2-Tsynth24
pMM1303	pRSII406-EcoRI-pGPD-SpyCas9_NLS-ADH1t-XbaI
pMM1321	pRSII406-pGPD-ATG-XbaI-yo_NLS_d _{16A} Nme1Cas9-SalI-GGTGGA-TAA(STOP)-CYC1t
pMM1322	pRSII406-pGPD-ATG-XbaI-yo_NLS_Nme1Cas9-SalI-GGTGGA-TAA(STOP)-CYC1t
pMM1424	pRSII406-pGPD-ATG-XbaI-yo_d _{16A} Nme1Cas9-GS-HAtag-SalI-TAA-CYC1t
pMM1325	pRSII405-pSNR52-sgRNA1_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1349	pRSII405-pSNR52-sgRNA2_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1326	pRSII405-pSNR52-sgRNA3_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1350	pRSII405-pSNR52-sgRNA4_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1327	pRSII405-pSNR52-sgRNA5_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1351	pRSII405-pSNR52-sgRNA6_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1381	pRSII405-pSNR52-random sequence(24nt)_DR(Nme1Cas9)-SUP4t
pMM1328	pRSII405-pSNR52-sgRNA1_yEBFP2_DR(SpyCas9)-SUP4t
pMM1352	pRSII405-pSNR52-sgRNA2_yEBFP2_DR(SpyCas9)-SUP4t
pMM1329	pRSII405-pSNR52-sgRNA3_yEBFP2_DR(SpyCas9)-SUP4t
pMM1353	pRSII405-pSNR52-sgRNA4_yEBFP2_DR(SpyCas9)-SUP4t
pMM1330	pRSII405-pSNR52-sgRNA5_yEBFP2_DR(SpyCas9)-SUP4t
pMM1354	pRSII405-pSNR52-sgRNA6_yEBFP2_DR(SpyCas9)-SUP4t
pMM1402	pRSII405-pSNR52-random sequence(20nt)_DR(SpyCas9)-SUP4t
pMM1445	pRSII405-pSNR52-sgRNAr1_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1428	pRSII405-pSNR52-sgRNAr2_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1429	pRSII405-pSNR52-sgRNAr3_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1453	pRSII405-pSNR52-sgRNAr4_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1426	pRSII405-pSNR52-sgRNAr5_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1446	pRSII405-pSNR52-sgRNAr6_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1447	pRSII405-pSNR52-sgRNAr7_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1430	pRSII405-pSNR52-sgRNAr8_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1448	pRSII405-pSNR52-sgRNAr9_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1427	pRSII405-pSNR52-sgRNAr10_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1454	pRSII405-pSNR52-sgRNAr11_yEBFP2_DR(Nme1Cas9)-SUP4t

pMM1455	pRSII405-pSNR52-sgRNAr12_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1431	pRSII405-pSNR52-sgRNAr13_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM1380	pRSII405-pSNR52-sgRNAr14_yEBFP2_DR(Nme1Cas9)-SUP4t
pMM728	pRSII405-Tsynth8.1_pCYC1noTATA-yEGFPgg-CYC1t_atc
pMM813	pRSII406-pGPD-ATG-NLS-GS-HIStag-GS-yo_LwaCas13a-GS-NLS-TAA-CYC1t
pMM843	pRSII406-pGPD-ATG-NLS-GS-HIStag-GS-yo_RfxCas13d-GS-NLS-TAA-CYC1t
pMM805	pRSII404-pSNR52-sgRNAr1_yEGFPgg_DR(LwaCas13a)-SUP4t
pMM806	pRSII404-pSNR52-sgRNAr2_yEGFPgg_DR(LwaCas13a)-SUP4t
pMM933	pRSII404-pSNR52-sgRNAr3_yEGFPgg_DR(LwaCas13a)-SUP4t
pMM888	pRSII404-pSNR52-sgRNAr4_yEGFPgg_DR(LwaCas13a)-SUP4t
pMM673	pRSII404-pSNR52-RL-s(79)-RL-s(116)-RL-s(265)-RL-s(79)-RL-s(116)-RL-s(265)-SUP4t (RL: Repeat LwaCas13a; s: spacer)
pMM792	pRSII424-pSNR52-sgRNAr1_yEGFPgg_DR(LwaCas13a)-SUP4t
pMM793	pRSII424-pSNR52-sgRNAr2_yEGFPgg_DR(LwaCas13a)-SUP4t
pMM934	pRSII424-pSNR52-sgRNAr3_yEGFPgg_DR(LwaCas13a)-SUP4t
pMM889	pRSII424-pSNR52-sgRNAr4_yEGFPgg_DR(LwaCas13a)-SUP4t
pMM839	pRSII404-pSNR52-sgRNAr1_yEGFPgg_DR(UrCas13d)-SUP4t
pMM840	pRSII404-pSNR52-sgRNAr2_yEGFPgg_DR(UrCas13d)-SUP4t
pMM935	pRSII404-pSNR52-sgRNAr3_yEGFPgg_DR(UrCas13d)-SUP4t
pMM890	pRSII404-pSNR52-sgRNAr4_yEGFPgg_DR(UrCas13d)-SUP4t
pMM841	pRSII424-pSNR52-sgRNAr1_yEGFPgg_DR(UrCas13d)-SUP4t
pMM842	pRSII424-pSNR52-sgRNAr2_yEGFPgg_DR(UrCas13d)-SUP4t
pMM936	pRSII424-pSNR52-sgRNAr3_yEGFPgg_DR(UrCas13d)-SUP4t
pMM891	pRSII424-pSNR52-sgRNAr4_yEGFPgg_DR(UrCas13d)-SUP4t
pMM1062	pRSII404-pSNR52-sgRNAr1_yEGFPgg_RfxCas13d(optimal DR)-SUP4t
pMM1064	pRSII404-pSNR52-sgRNAr2_yEGFPgg_RfxCas13d(optimal DR)-SUP4t
pMM995	pRSII404-pSNR52-sgRNAr3_yEGFPgg_RfxCas13d(optimal DR)-SUP4t
pMM1063	pRSII404-pSNR52-sgRNAr1_yEGFPgg_RfxCas13d(standard DR)-SUP4t
pMM1065	pRSII404-pSNR52-sgRNAr2_yEGFPgg_RfxCas13d(standard DR)-SUP4t
pMM997	pRSII404-pSNR52-sgRNAr3_yEGFPgg_RfxCas13d(standard DR)-SUP4t
pMM1022	pRSII403-pADH1-ATG-Rsp_WYL1(with HAtag)-NLS-GS-NLS-TAA-CYC1t_atc
pMM1023	pRSII403-pGPD-ATG-Rsp_WYL1(with HAtag)-NLS-GS-NLS-TAA-CYC1t_atc

Table S2. DNA sequences used in this work.

NAME	SEQUENCE
pTEF2	GCTACCTATATCCACCATAACATCAATCATCGGGTGTGGTATTTACCAATAATG TTAATGTATATATATATATGGGCCGTACTTACATAGTAGATGTCAAGCG TAGGCCTCCCGTGTGAGGGGCCATAACCAAGGTATCTATAGACCGC CAATCAGCAAACCTACCTCGTACATTACATGTTGACCCCACACATTATACACCCAGAC CGCGACAAATTACCCATAAGGTTGTTGTGACGGCGTGTACAAGAGAACGTGGG AACTTTAGGCTCACCAAAAAAGAAAGAAAAACGAGTTGCTGACAGAACGCC TCAAGAAAAAAAATTCTTCGACTATGCTGGAGGCAGAGATGATCGAGCCGG TAGTTAACTATATAGCTAAATTGGTCCATCACCTCTTTCTGGTGTGCTCCTTCT AGTGCTATTTCTGGCTTTCCATTCCCCCTCTCTCTCTCTCTCTCTCTCT ATAAATTCTCTTGCACTTCTATTTCTCTCTATCTACTTGTTATTCCCTCAA GGTTTTTTAAGGAGTACTTGTTTAAATACGGTCAACGAACACTATAATTAAAC TAAAC
pGPD	CAGTCGAGTTATCATTATCAAACTGCCATTCAAAGAATACGTAATAATTAAAG TAGTGATTTCTAACTTTATTAGTCAAAAAATTAGCCTTTAATTCTGCTGTAACCC GTACATGCCAAAATAGGGGGCGGGTACACAGAAATATAACATCGTAGGTGCTG GGTGAACAGTTATTCCCTGGCATCCACTAAATATAATGGAGCCGCTTTAAGCTG GCATCCAGAAAAAAAAGAATCCCAGCACCAAAATTGTTTCTCACCAACCAC AGTCATAGGTCCATTCTTAGCGCAACTACAGAGAACAGGGGCACAAACAGGCA AAAAACGGGCACAAACCTCAATGGAGTGATGCAACCTGCCTGGAGTAAATGAC ACAAGGCAATTGACCCACGCATGTATCTCATTTCTACACCTTCTATTACCTTC TGCTCTCTGATTGGAAAAGCTGAAAAAAAGGTTGAAACCAGTCCCTGAAA TTATCCCCACTTGACTAATAAGTATATAAGACGGTAGGTATTGATTGATTCTGT AAATCTATTTCTAAACTCTTAAATTCTACTTTATAGTTAGTCTTTTTAGTTTA AAACACCAAGAACCTAGTTCGAATAAACACACATAAACAAACAAA
pADH1	CAGTCGAGTTATCATTATCAAACTGCCATTCAAAGAATACGTAATAATTAAAG TAGTGATTTCTAACTTTATTAGTCAAAAAATTAGCCTTTAATTCTGCTGTAACCC GTACATGCCAAAATAGGGGGCGGGTACACAGAAATATAACATCGTAGGTGCTG GGTGAACAGTTATTCCCTGGCATCCACTAAATATAATGGAGCCGCTTTAAGCTG GCATCCAGAAAAAAAAGAATCCCAGCACCAAAATTGTTTCTCACCAACCAC AGTCATAGGTCCATTCTTAGCGCAACTACAGAGAACAGGGGCACAAACAGGCA AAAAACGGGCACAAACCTCAATGGAGTGATGCAACCTGCCTGGAGTAAATGAC ACAAGGCAATTGACCCACGCATGTATCTCATTTCTACACCTTCTATTACCTTC TGCTCTCTGATTGGAAAAGCTGAAAAAAAGGTTGAAACCAGTCCCTGAAA TTATCCCCACTTGACTAATAAGTATATAAGACGGTAGGTATTGATTGATTCTGT AAATCTATTTCTAAACTCTTAAATTCTACTTTATAGTTAGTCTTTTTAGTTTA AAACACCAAGAACCTAGTTCGAATAAACACACATAAACAAACAAA
pSNR52	CTTGAAAAGATAATGTGATTATGCTTCACTCATATTATACAGAAACTTGATGTT TTCTTTCGAGTATATACAAGGTGATTACATGTACGTTGAAGTACAACCTAGATTT GTAGTGCCTCTGGCTAGCGTAAAGGTGCGCATTTTACACCCCTACAATGTT CTGTTCAAAGATTGGTAAACGCTGTAGAAGTGAAGTTGGTGCATGTT GGCGTTGAAACTCTCCGAGTGAAGATAATGATC

DEG1t_pCYC1n oTATA	AATAATATATAAACCTGTATAATATAACCTGAAGACTATTTCTTCTtcTTCCCTTAT ACATtAGGACCTTGCAAGCATAAAATTACTATACTTCTATAGACACACAAACACAAATA CACACACTAAATTATA
Tsynth8.1_pCY C1noTATA	TTTCCCTATATAAAACTCATTTACTTATGTAGGAATAAAGAGTATCATCTTCAAATTCTT TCCTTACATTAGGACCTTGCAAGCATAAAATTACTATACTTCTATAGACACACAAACA CAAATACACACACTAAATTATA
yEBFP2	atgGTTCCAAGGGTGAAGAATTATTACCCGGTGTGTCCTATTTGGTGAATTGG ACGGTGATGTCAACGGTCACAAGTTCTCGTTAGAGGTGAAGGTGAAGGTGACGC TACCAACGGTAAGTTGACCTTGAAGTTCATCTGTACCACTGGTAAGTTGCCGTGTTCC TTGGCCAACTTGGTTACGACTTGTCTACGGTGTCAATGTTGCCCGTTACCC AGATCACATGAAGCAACATGATTCTTAAATCGCCATGCCAGAAGGTTACGTTCA AGAAAGAACTATCTTCTCAAGGACGATGGTACTTACAAAAGTAGAGCCGAAGTCA AGTTGAAAGGTGATACTTAGTCAATAGAATGAAATTGAAGGGTGTGATTCAAG GAAGATGGTAACATTTGGGTACAAGTTAGAATACAATTCAACTCCCATAATATT ACATTATGGCTGTTAAGCAAAGAACGGTATTAAGGTCAACTTAAATCAGACACA ACGTTGAAGACGGTCTGTCCAATTAGCTGACCACCTACCAACAAAACACTCCAATTG GTGACGGTCCAGTCTTATTACAGATTCTCATTACTGTCTACCCAATCTGCTTGTCT AAGGACCCAATGAAAAGAGAGATCACATGGTCTGTTAGAATTCTGACTGCTGC TGGTATCACTTGGGTATGGACGAATTGTACAAGtaa
yEGFPgg	atgTCTAAAGGTGAAGAATTATTCACTGGTGTGTCCTAATTGGTGAATTAGATG GTGATGTTAATGGTCACAAATTCTGTCTCCGGTGAAGGTGAAGGTGATGCTACTT ACGGTAATTGACCTTAAATTATTGACTACTGGTAAATTGCCAGTCCATGGCC AACCTTAGTCACTACTTCGGTTATGGTGTCAATGTTGCCAGAAGGTTATGTTCAAGAAAG ATGAAACAACATGACTTTCAAGTCTGCCATGCCAGAAGGTTATGTTCAAGAAAG AACTATTTTTCAAGATGACGGTAACTACAAGACCAGAGCTGAAGTCAAGTTG AAGGTGATACTTAGTTAATAGAATGAAATTAAAGGTATTGATTAAAGAACGATG GTAACATTTAGGTACAATGGAAATACAACACTATACTCTCACAAATGTTACATCAT GGCTGACAAACAAAAGAATGGTACAAAGTTAACTTCAAAATTAGACACAACATTG AAGATGTTCTGTTCAATTAGCTGACCATTCAACAAAACACTCCAATTGGTGTG TCCAGTCTGTTACAGACAACCAATTACTTCACTCAATCTGCCATTCAAAGATC CAAACGAAAAGAGGACCATGGTCTGTTAGAATTGTTACTGCTGCTGGTATTA CCCATGGTATGGATGAATTGTACAAAtaa
yo_SpyCas9	atgGACAAGAAGTATTCTATCGGACTGGACATGGACTAATAGCGTCGGGTGGC CGTCATCACTGACGAGTACAAGGTGCCCTCAAGAAGTTCAAGGTGCTCGGGAAACA CCGACCGGCATTCCATCAAGAAAAATCTGATCGGAGCTCTCCTCTTGATTCAAGGG AGACCGCTGAAGCAACCGCCTCAAGCGGACTGCTAGACGGCGGTACACCAGGA GGAAGAACCGGATTGTTACCTCAAGAGATATTCTCAACGAAATGGCAAAGGTC GACGACAGCTTCTCCATAGGCTGGAAGAATCATTCTCGTGGAGAGGATAAGAA GCATGAACGGCATCCATCTCGTAATATCGTCACGAGGGTGGCTATCACGAGAA ATACCCAACCATCTACCATCTTCGCAAAAAGCTGGTGGACTCAACCGACAAGGCAG ACCTCCGGCTTATCTACCTGGCCCTGGCCCACATGATTAAGTTCAAGGGCCACTTCC TGATCGAGGGCGACCTCAATCCTGACAATAGCGATGTGGATAAACTGTTCATCCAGC TGGTGCAGACTTACAACCAGCTTGAAGAGAACCCATCAATGCAAGCGGAGTC GATGCCAAGGCCATTCTGTCAGCCGGCTGTCAGAGCGCAGACTTGAGAATCT TATCGCTCAGCTGCCGGTGAAGAGAACCCATCAATGCAAGCGGAGTC TTCACTGGGCTGACTCCCAATTCAAGTCTAATTGACCTGGCAGAGGATGCCA AGCTGCAACTGTCCAAGGACACCTATGATGACGATCTGACAACCTCCTGGCCAG

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yo_dNme1Cas9	atgGCTGCTTTAAACCAAATTCTATTAAATTATTTGGGTTGGCTATTGGTATTGCT TCTGTTGGTGGCTATGGTGAATCGATGAGGAAGAAAATCCAATTAGATTGATT GATTTGGGTGTCAGAGTTTGAAAGAGCTGAAGTCAAAGACTGGTATTCTT GGCTATGGCTAGAAGATTGGTAGATCTGTTAGAAGATTGACTAGAAGGAGGGCTC ATAGATTGTTGAGAAGTCTAGAAGATTGAAAGAGAAGGTGTTGCAAGCTGCT AATTTGATGAAAATGGTTGATTAAATCTTGCCAAACTCCATGGCAATTGAGA GCTGCAGCTTGGATAGAAAATTGACTCCTTGGATGGTCTGCTGTTGTCAT TTGATTAAACATAGAGGTTATTGTCCTCAAAGAAAAATGAAGGTGAAACTGCTGAT AAAGAATTGGGTGCTTGTTGAAGGGTGTGCTGGAATGCTCATGCTTGCAC TGGTATTAGAACCTCTGCTGAATTGGCTTGAATAAATTGAAAAAGAATCTGG TCATATTAGAAATCAAAGATCTGATTACTCACATACTTTCTAGGAAGGATTGCAA GCTGAATTGATTATTGTTGAAAAACAAAAGAATTGTAATCCACATGTTCTG GTGGTTGAAAGAAGGTATTGAAACTTGTGATGACTCAAAGACCTGCTTGTCT GGTGTGCTGTTCAAAAATGTTGGTCATTGACTTTGAACCTGCTGAACCAAAA GCTGCTAAAACACATATACTGCTGAAAGGTTATTGGTAACTAAGTGAATAATT TGAGAATTGGACAAGGTTCTGAAAGACCATTGACTGATACTGAAAGAGCTACA TTGATGGATGAACTTATAGGAAATCTAAATTGACTTATGCTCAAGCTAGAAAATT TAGGTTGGAAGACTGCTTTTAAAGGTTGAGATATGTAAGATAACGCAG AAGCTTCACTTGATGGAAATGAAAGCTTATCATGCTATTCAAGAGCTTGGAAA AAGAAGGTTGAAAGATAAAAATCTCATTGAATTGTCCTGAAATTGCAAGATG AAATTGGTACAGCTTTCTTGTAAAGACAGATGAAGATAATTGTTGAGATTGA AAGATAGAATTCAACCTGAAATTAGAACGATTGTAACATCTCATTGATAA ATTGTTCAAATTCTTGAAAGCTTGAGAAGAATTGTTCCATTGATGGAAACAAG TAAAAGATATGATGAAGCATGTGCTGAAATTATGGTGTATTGTAAGGAAAAAAA CACTGAAGAAAAATTATTGCCACCAATTCTGCTGATGAAATTAGAAATCCTGT TGTTTGAGAGCTTGTACAAGCAAGAAAAGTTATCAATGGTGTGTTAGAAGAT ATGGTCTCTGCTAGAATTGAAACTGCAAGGGAGGGTGGTAAGTCTTTA AAGATAGAAAGGAAATTGAAAAAGACAAGAAGAGATAAGAAAAGACAGAGAAA AAGCTGCAGCTAATTAGGAATACTTCTAACTTCGTCGGTAACCCAAATCTA AGGACATTGAAATTGAGATTGATGAAACAACACCGAAAATGTTGATTCTG GTAAAGAGATCAATTAGGAAGATTGAAATGAAAAAGGTTATGTTGAAATTGATGCT GCTTGCCTTCAAGAACTTGGGATGACTCTTTAATAATAAGTTGGTTGG GTTCTGAAAACAAAATAAAGGTAATCAAACCTCTTGAATATTAAACGTAAGG ATAATTCAAGAGAATGGCAAGAATTAAAGCTAGAGTTGAAACATCAAGATTCCA AGATCTAAGAAACAAAGAATTGTTGCAAGAGTTGACGAGGATGGTTAAAGA GAGAAACTAAACGACACTAGATACTGTTAATAGATTGTCATTGCTGAT AGAATGAGATTGACTGGTAAGGTAAGGAGTTGCTTCAATTGCTGAAAT TACTAATTGTTGAGAGGTTGGGTTGAGAAAAGTTAGAGCTGAAATGATA GACATCATGCTTGGATGCTGTTGCTGTTCAACTGTTGCTATGAGCAGCAA AAATTACTAGGTTGTTAGATATAAAGAGATGAATGTTGATGGTAAACTATTGA TAAAGAAAATGGTAAGTTGATCAGGAAACTCATTCTCAACCATGGAAATT TTTGCTCAAGAAGTTGATTAGAGTTGAAACCTGATGGTAAGCCTGAATT TGAAGAAGCTGATACTTGGAAAATTGAGAACTTGTGTTGGCTGAAAATTGCTT CAAGACCTGAAGCTGTTCATGAAATGTTACTCCATTGTTGTTCAAGGGCTCAA ATAGAAAATGTCTGGTAAGGTCACATGGAAACTGTTAAATCTGCTAAAGATTG GATGAAGGTGTTCTGTTGAGGGTCCATTGACACAAATTGAAAGATTG GAAAAAAATGGTCAATAGAGAAAGAGAACCAAAATTGATGAAAGCTTGAAGCTA

	GATTGGAAGCTATAAAGATGATCCTGCTAAAGCTTTGCTGAACCATTATAAATA TGATAAAGCTGGTAATAGAACTCAACAAGTAAAGCTTAGAGTTGAACAAGTTC AAAAAACTGGTGTGTTGGGTTAGAAATCATATGGTATTGCTGATAATGCTACTATGGT TAGAGTTGATGTCTTGAAAAGGAGATAAAATATTACTTGGTCCAATTATTCTTGG CAAGTTGCTAAAGGTATTTGCCGATAGAGCTGTTCAAGGTAAAGACGAAGA AGACTGGCAGTTGATTGATGATTCTTTAACCTTAATTTCTTGATCCTAACGAT TTGGTGAAGTCATTACTAAAAAGCTAGAATGTTGGTTATTTGCATCTGTCACA GAGGTACTGGTAACATTAATCAGAATTGATGATTGGATCATAAAATCGTAAGAA TGGTATTGGAGGTATTGGTAAACTGCTTGTCTTCAAAAATATCAAATT GACGAATTGGAAAAGAAATTAGGCCATGTAGATTGAAAAAAAGACCACCTGTTA GAtaa
yo_d _{16A} Nme1Cas9 (The marked <u>cat</u> is the A588H mutation with respect to yo_dNme1Cas9)	atgGCTGCTTTAACCAAATTCTATTAAATTATATTTGGGTTGGCTATTGGTATTGCT TCTGTTGGTGGGCTATGGTGAATCGATGAGGAAGAAAATCCAATTAGATTGATT GATTTGGGTGTCAGAGTTTGAAAGAGCTGAAGTCCAAAGACTGGTATTCTT GGCTATGGCTAGAAGATTGGCTAGATCTGTTAGAAGATTGACTAGAAGGAGGGCTC ATAGATTGTTGAGAAGACTAGAAGATTATTGAAGAGAGAAGGTGTTTGCAAGCTGCT AATTTGATGAAAATGGTTGATTAAATCTTGCCAAACTCCATGGCAATTGAGA GCTGCAGCTTGGATAGAAAATTGACTCCTTGGATGGTCTGCTGTTGTTGCAT TTGATTAAACATAGAGGTATTGCTCAAGAAAAATGAAGGTGAAACTGCTGAT AAAGAATTGGGTGCTTGTGAAGGGTGTGCTGGAATGCTCATGCTTGCAAAC TGGTATTAGAACTCCTGCTGAATTGGCTTGAATAAATTGAAAAAGAATCTGG TCATATTAGAAATCAAAGATCTGATTACTCACATACTTTCTAGGAAGGATTGCAA GCTGAATTGATTATTGTTGAAAACAAAAGAATTGGTAATCCACATGTTCTG GTGGTTGAAAGAAGGTATTGAAACTTGTGATGACTCAAAGACCTGCTTGTCT GGTGATGCTGTTCAAAAATGTTGGGTCTTGAACCTGCTGAACCAAAA GCTGCTAAAACACATATACTGCTGAAGGTTTATTGGTAACTAAGTTGAATAATT TGAGAATTGGAAACAAGGTTCTGAAAGACCAATTGACTGATACTGAAAGAGCTACA TTGATGGATGAACCTTATAGGAAATCTAAATTGACTTATGCTCAAGCTAGAAAATT TAGGTTGGAAAGATACTGCTTTTAAAGGTTGAGATATGGTAAAGATAACGCAG AAGCTTCTACTTGTATGGAAATGAAAGCTTATCATGCTATTCAAGAGCTTGGAAA AAGAAGGTTGAAAGATAAAAATCTCATTGAATTGTCCTGAATTGCAAGATG AAATTGGTACAGCTTTCTTGTTAAGACAGATGAAGATATTACTGGTAGATTGA AAGATAGAATTCAACCTGAAATTAGAAGCATTGAAACATATCTCATTGATAA ATTGTTCAAATTCTTGTTGAAGCTTGAGAAGAATTGTCATTGATGGAAACAAGG TAAAAGATATGATGAAGCATGTGCTGAATTATGGTATGATTGGTAAAGGGAAA CACTGAAGAAAAATTATTGCCACCAATTCTGCTGATGAAATTAGAAATCCTGT TGTTTGAGAGCTTGTCACAAGCAAGAAAAGTTATCAATGGTGTGTTAGAAGAT ATGGTTCTCCTGCTAGAATTGAAACTGCAAGGGAGGGTGGTAAGTCTTTA AAGATAGAAGGAAATTGAAAAAGACAAGAAGAGAAATTAGAAAAGACAGAGAAA AAGCTGCAGCTAAATTAGGAATACTTCTAACTCGTCGGTAACCAAAATCTA AGGACATTGAAATTGAGATTGTATGAACAACAACACGGAAAATGTTGATTCTG GTAAAGAGATCAATTAGGAAGATTGAATGAAAAAGGTTATGTTGAAATTGAT <u>catG</u> CTTGCCATTTCAGAACTGGGATGACTCTTAAATAATAAAGTTGGTTGGG TTCTGAAAACCAAAATAAGGTAATCAAACCTCTTGAATATTAAACGGTAAGGAT AATTCAAGAGAATGGCAAGAATTAAAGCTAGAGTTGAACATCAAGATTCCAAG ATCTAAGAAACAAAGAATTGTTGTCAGAAGTTGACGAGGATGGTTAAAGAGA GAAACTAAACGACACTAGATACGTTAATAGATTGTCATTGCTGATAG AATGAGATTGACTGGTAAAGGTAAGGAGGTTGCTTCAATTGTCATAAGGTC TAATTGTTGAGAGGTTGGGTTGAGAAAAGTTAGAGCTGAAAATGATAGAC ATCATGCTTGGATGCTGTTGCTGTTCAACTGTTGCTATGCAGCAAAAT TACTAGGTTGTTAGATATAAAGAGATGAATGCTTGTGTTGAAACTATTGATAAA GAAACTGGTGAAGTTGATCAGAAACTCATTTCCTCAACCATTGGGAAATT

	GCTCAAGAAGTTATGATTAGAGTTTGGAAAACCTGATGGTAAGCCTGAATTGA AGAAGCTGATACTTGGAAAAATTGAGAACCTTGTGGCTAAAAATTGCTTCAA GACCTGAAGCTTCAATGTTACTCCATTGTTCAAGGGCTCAAATAG AAAAATGTCGGTCAAGGTACATGGAAACTGTTAACTGCTAAAAGATTGGATG AAGGTGTTCTGTTGAGGGTCCATTGACACAATTGAAATTGAAAGATTGGAA AAAATGGTCAATAGAGAAAGAGAACCAAATTGTATGAAGCTTGAACAGCTAGATT GGAAGCTCATAAAGATGATCCTGCTAAAGCTTGTGATAATGCTACTATGGTTAGA AAAGCTGGTAATAGAACTCAACAAGTTAAAGCTGTTAGAGTTGAAACAAGTCAAA AACTGGTGTGGGTTAGAAATCATAATGGTATTGCTGATAATGCTACTATGGTTAGA GTTGATGTCTTGAAAAGGAGATAAATATTACTTGGTCCAATTATTCTGGCAAG TTGCTAAAGGTATTGCCTGATAGAGCTGTTCAAGGTAAAGACGAAGAAGAC TGGCAGTTGATTGATCTTTAACTTTAAATTCTTGCATCCTAACGATTGGT TGAAGTCATTACTAAAAAGCTAGAATGTTGGTATTTCATCTGTCACAGAGG TACTGGTAACATTAATATCAGAATTGATCATAAAATCGGTAAAGATGGT ATTGGAAAGGTATTGGTAAACTGTTCTTCAAAAATATCAAATTGACG AATTGGAAAAGAAATTAGGCCATGTAGATTGAAAAAGACCACCTGTTAGAtaa
yo_Nme1Cas9 (The marked <u>gat</u> and <u>cat</u> are the A16D and A588D mutations with respect to yo_dNme1Cas9)	atgGCTGCTTTAACCAAATTCTATTAAATTATATTTGGGTTT <u>gat</u> ATTGGTATTGCT TCTGTTGGTGGGCTATGGTGAATCGATGAGGAAGAAAATCCAATTAGATTGATT GATTGGGTGTCAAGAGTTTGAAGAGACTGAAGTTCAAAGACTGGTATTCTT GGCTATGGCTAGAAGATTGGTAGATCTGTTAGAAGATTGACTAGAAGGAGGGCTC ATAGATTGTTGAGAACCTAGAAAGATTATTGAAGAGAGAACGGTGTGCAAGCTGCT AATTGATGAAAATGGTTGATTAATCTTGCCTAAACTCCATGGCAATTGAGA GCTGCAGCTTGGATAGAAAATTGACTCCTTGGATGGTCTGCTGTTGTCAT TTGATTAACATAGAGGTTATTGTCTCAAAGAAAAATGAAGGTGAAACTGCTGAT AAAGAATTGGGTGCTTGTGAAGGGTGTGCTGGTAATGCTCATGCTTGCACAC TGGTGATTTAGAACCTCCTGTAATTGGCTTGAATAAATTGAAAAAGAATCTGG TCATATTAGAAATCAAAGATCTGATTACTCACATACTTTCTAGGAAGGATTGCAA GCTGAATTGATTTATTGTTGAAAACAAAAGAATTGGTAATCCACATGTTCTG GTGGTTGAAAGAAGGTATTGAAACTTGTGATGACTCAAAGACCTGCTTGTCT GGTGATGCTGTTCAAAAATGTTGGGTATTGACTTTGAACCTGCTGAACCAAAA GCTGCTAAAACACATACTGTAAGGTTATTGGTTACTAAGTTGAATAATT TGAGAATTGGAAACAAGGTTCTGAAAGACCATTGACTGATACTGAAAGAGCTACA TTGATGGATGAACTTATAGGAAATCTAAATTGACTTATGCTCAAGCTAGAAAATT TAGGTTGGAAAGATACTGCTTTTAAAGGTTGAGATATGGTAAAGATAACGCAG AAGCTTCACTTGATGAAATGAAAGCTTATCATGCTATTCAAGAGCTTGGAAA AAGAAGGTTGAAAGATAAAAATCTCATTGAATTGTCCTGAATTGCAAGATG AAATTGGTACAGCTTTCTTGTTAAGACAGATGAAGATATTACTGGTAGATTGA AAGATAGAATTCAACCTGAAATTAGAAGCATTGTAACATATCTCATTGATAA ATTGTTCAAATTCTTGAAAGCTTGAAGAAGATTGTTCCATTGATGGAACAAGG TAAAAGATATGATGAAAGCATGCTGAAATTATGGTAGATTGATCATTGGTAAAAAAA CACTGAAGAAAAATTATTGCCACCAATTCTGCTGATGAAATTAGAAATCCTGT TGTTTGAGAGCTTGTACAAGCAAGAAAAGTTATCAATGGTGTGTTAGAAGAT ATGGTTCTCTGCTAGAATTCTGAAACTGCAAGGGAGGGTGGTAAGTCTTTA AAGATAGAAGGAAATTGAAAAAGACAAGAAGAGAAATGAAAAGACAGAGAAA AAGCTGCAGCTAAATTAGGAATACTTCTAACTTCGTCGGTAACCAAAATCTA AGGACATTGAAATTGAGATTGATGAAACAACACCGAAAATGTTGATTCTG GTAAAGAGATCAATTAGGAAGATTGAAATGAAAAAGGTTATGTTGAAATTGAT <u>catG</u> CTTGGCCATTTCAGAACCTGGGATGACTCTTTAATAATAAAAGTTGGTTGGG TTCTGAAAACCAAAATAAGGTAATCAAACCTCTTGAATATTAAACGGTAAGGAT AATTCAAGAGAATGGCAAGAATTAAAGCTAGAGTTGAAACATCAAGATTCCAAG ATCTAAGAAACAAAGAATTGGTGTGCAAGAGTTGACGAGGATGGTTAAAGAGA GAAACTTAAACGACACTAGATACGTTAATAGATTGGTGTCAATTGTTGCTGATAG

	AATGAGATTGACTGGTAAAGGTAAAAAAGAGTTTGCTTCTAATGGTCAAATTAC TAATTGTTGAGAGGTTTGGGTTGAGAAAAGTAGAGCTGAAAATGATAGAC ATCATGTTGGATGCTGTTGCTGTTCAACTGTTGCTATGCAGAAAAAT TACTAGGTTGTTAGATATAAAGAGATGAATGCTTTGATGGTAAACTATTGATAAA GAAACTGGTGAAGTTGATCAGAAAAACTCATTTCCTCAACCAGGGAAATT GCTCAAGAAGTTGATTAGAGTTTGAGAACCTGATGGTAAAGCCTGAATT AGAAGCTGATACTTGAGAAAATTGAGAACCTGTTGGCTGAAAGATTGAA AAAATGGTCAATAGAGAAAGAGAACCAAAATTGATGAAGCTTGAAGCTAGATT GGAAGCTCATAAAGATGATCCTGCTAAAGCTTGTGTAACCAATTATAATGAT AAAGCTGGTAATAGAACTCAACAAGTTAGCTGTTAGAGTTGAACAAGTTCAA AACTGGTGGGGTAGAAATCATAATGGTATTGCTGATAATGCTACTATGGTAA GTTGATGTCGGAGGAGATAAATATTACTGTTCCAATTATTCTGGCAAG TTGCTAAAGGTATTGCTGATAGAGCTGTTGTTCAAGGTAAGACGAAGAAC TGGCAGTTGATTGATGATTCTTAACTTTAAATTCTTGCATCCTAACGATTGGT TGAAGTCATTACTAAAAAGCTAGAATGTTGGTTATTGCTGATCTGTACAGAGG TACTGGTAACATTAATATCAGAATTGATGATTGGATCATAAAATCGGTAAAGATT ATTGGAGGTTAGGGTAAACTGCTTGTCTTCAAAATATCAAATTGACG AATTGGAAAAGAAATTAGGCCATGTAGATTGAAAAAGACCACCTGTTAGAtaa
yo_LwaCas13a	atgAAAGTTACAAAGGTGACGGAAATTCAACACAAAAATATATTGAAGAGGGTA AATTGGTTAAGTCACCTCTGAGGAAACAGAACCTCAGAAAGATTGCTGAATT GTTGCTATAAGGTAGATATATACATTAAGAACATCCAGAACACGCTCAGAGGAA GAAAACAGAATTAGGAGAGAGAACATTGAAAAAATTCTCTAATAAAAGTTTAC ATTGAAAGACTCTGCTTGACTTGAGAACAGAACAGGAGAAAATGCAGTCCA AGATAAGAACTATTCTGAGAGGATATTGAGTACGATTGAAAAAATAAAAAC TCTTTTCAGTTGAAGAAAATTGTTGAATGAAGACGTTAATTGAGGAATT GGAAATTAGAAAAGATGTTGAAGCAAATTGAAACAAATTAAATTCTTAAAT ATTCTTGAGGAAATAAGCTAACTACCAAAAATTAAACGAAAACAATGTTGA AAAAGTTGGTGGAAATCAAAGAGGAATTATTACGATTATTACAGAGAAC GCTAACAGAACATTGGAAACTACCCATAAGATTATTGGTAGGAAAATGACAAA GAAAATTGCTAAGATTATTGAGAACATTCAAATGTTAATAATTAAAGA ATTGATTGAAAAATTCCAGATATGTCAGAATTGAAAAAGTCACAAGTTCTACA AAACTACTTGGATAAGAACATTGAAATGACACAAAATTAAAGTACGCATT TCACCTCGTTGAAATTGAGATGTCATTGTTGAAAAACTATGTTATAAAAGGT TATCAAATATATCTAATGATAAGATTAAAAGAACATTGAGTACCAAAATTGAG AAATTAAATTGAAAACAAATTGTTGAATAAATTGGATACTTATGTCAGAAATTGCG GTAAGTATAATTACTTGAGGTTGGTGAATTGCTACATCTGATTGCT AGAAATAGACAAAACGAAGCTTCTGAGAAATATAATTGGTGTCTCATGTTG TTATTCTCATTAAGAAATTAGAACATTGAGAACAGACATTACTGGAA GGATGAGGGTAAACTGTTAAAACAACAAAGGTGAAGAAAAGTATGTTG GAGAGGTTGACAAGATTACAATGAAAATAGCAAATGAAGTTAAAGAGAATT TAAAATGTTACTTACGATTCAACATGGACACAAAGAACATTGAGAT TTTCGCAAACATTGACGAAGCTATTCTTCTATTAGACACGGTATAGTTCACTT AACTTAGAATTGGAAGGTAAAGACATATTGCTATTAAAACATAGCTCCTCAG AGATTCTAAGAAAATTGTTGAGAACATAACGAAAAGAAATTAAAATTAAA AATATTAAAGCAATTAAATTGCTAATGTTAATTACTACGAAAAGATGTCAT TATTAATATTGAAAACACAAAATTAAATTGTTAATAAAAACATACCAATTG TCCATCTTACTAAATTATAACAAAATAGAACATTGAGAAATTGAGTT

	CTTTGGTCTGTC AAGAATATATTATGGAGAGTT TTCAAAATTACTAATGAAGTT GGTCATTATAAGTACCAGAAGTT ACTTAGCTATAATTCAATCA AAATACATACATTGATT GAATAAGAACAACTTGAAGT ATTTCTCAAAAATT AAAAAATTACGAGAAGC ATTCTAGAGAATT ATGTTTATTGATT TTCTTAGAAAAT TAATTAAACATAGAG GAGAAAATTGCT ACTCAAATAAG TAGGAAATACG AGAATACG GTTGAGTT ATTGGTTGGT GAATTTC CGTTAAAT TGTATAAG GTTAAACA ATACCTCAT TTGTCTT AAAGAGT ATTCAACAT AGTTAATG CGAATATAA atgGAAGCCAGT GTACCCCTTG GCTAGGCTG GAAGCTTTCT CTAAGTTTCT GGACCA GGACCA TTCGGTGA CTTGGACAT ATAACATC TACACCTAT ATGATAAG GATAACCC ACTACATC TTGAGGC TGGCTTATA CTTGTACG CCAACGT CTAAGTTG ACCACAAAG yo_RfxCas13d

	ATTTACAGATATTACATTGAGGAAGATGCCAAGGTTGCCGCTGCCAACAAATCTT GCCAGACAACGAAAAGAGTTGTCTGAGAAGGCATTTCGTTATCAACTTGAGA GGTTCTTCATGACGACCAGAAAGACGCCCTGTATTACGACGAGGCCAATAGAA TCTGGAGGAAATTGGAAAATATCATGCACAATATCAAAGAATTAGGGGAAATAA GACTAGAGAGTACAAGAAAAGGACGCCCTAGGCTCCTAGGATCTGCCAGCT GGAAGGGACGTTCTGCCTTCAGTAAATTGATGTACGCCCTGACTATGTTTTGGA TGGTAAGGAAATCAATGACCTTTGACTACTCTTATCAACAAATTGACAATATCC AAAGTTCTGAAGGTATGCCCTTATCGGTCAACGCTAAGTCGAGGAGA GTACGCCCTTTAAAGATTCTGCTAAATCGCTGATGAGCTTAGACTTATCAAAT CTTTGCCAGAATGGGAGGCCAATCGCTGATGCTAGGAGGGCTATGTATATCGA CGCCATTAGGATTCTGGTACTAACCTAGTTATGACGAGCTTAAAGCTTGGCG ACACCTCTCTTGTGATGAAAACGTAATAAAACTTAAAAGGGAAAACATGGAAT GAGAAATTATCATCAATAATGTCATCTAACAAAAGGTTCACTACTTGATTA GGTACGGAGATCCAGCCCCTTCACGAGATGCCAAGAACGGCCGTTGCAA GTCGCTTGGGAAGGATTGCCGACATCCAGAAGAACGCAAGGTCAGAACGGAAA GAACCAAGATCGACAGATACTACGAAACTTGTATTGGTAAAGATAAGGGTAAATCT GTCAGTGAAGGTCGATGCCCTGACCAAGATCATCACCGGTATGAACTACGATC AATTGACAAGAAGAGAAGTGTATTGAAGATACCGGAAGGGAGAACGCCGAAA GAGAAAAGTTCAAAAGATCATCTCTTACCTTACTGTTATCTACACATTGAA AAAACATCGTCAATATTAAATGCCAGATATGTCATCGGATTCCACTGCGTCGAGAG AGATGCCAGTTGATATAAGAAAAAGGTTACGACATTAAATCTTAAAAGTTGAA GAAAAGGGATTAGTGTACCGTAAAGCTTGTGAGGAGAGCCAAGGAGTCT CAGACAAAAGGAAGGACGTTGAAAAGAAATGGCGAGAGAGCCAAGGAGTCT ATCGACAGTTGGAGTCTGCCAACCAAAGTTGACGCTAATTATCAAGTACAG TGATGAAAAAAAGCCGAGGGAGTTCACTAGACAAATCAATAGAGAAAAGCCAA AACCGCCTGAATGTTACTTGAGGAATACCAAGTGGAACGTTATTACAGAGAA GATCTTTGAGAATCGATAATAAGACTTGTACCCCTTTAGAAACAAAGCTGTC CTTGGAGGTCGCTAGATACGCCACGCCATCACGACATTGCTGAGGTCAAT TCTTACTTCAGTTGATACGTTACCTACATTATGCAAGGAGTCAATTGAGAGATA CGAGAAAAGTTCTGGTAAAGTTCTGAATACTCGACGCCGTCACGATGAA AAAGTACAACGATAGACTCTAAATTGTTGTGTCCTTGTGAGGTTACTGATTCA AGATTTAGAATTGTCATCGAAGGCCCTTTGATAGAAACGAGGCTGCTAAGTT CGACAAGGAAAAAGAAAGTTAGTGGAAATAGTGGTTCTGGTaa
yo_RspWYL1 (with HAtag)	atgAAGATCGAGGAAGGAAAGGGTTATCCTTATGATGTTCTGATTATGCAATGCTTA TTCCCTCTCAACATTCTCTAAGCGCGACAAGAATGTACCTTATATTGCAAGAAGT TCAGTCGATACCGCTGTACCCCTAGCATATTCTGTAATCATAAAGGACAAGTCCATC TTTGAACCTTCACTTCACCTAATGGCTCTGTCTCCATGTCCTTCTGACCTCCAT CTTCGACAGCGCCTACATGCCCTCTAAAGTACAAGTCTGACGCCAATTACAAGTA CATAGGAATTCTCTTCTAACGCTTAAATGGCAGATAGAGGAGATCGACGA CGGCTTAGACGACAAGTCCAAAGAAATAATAAGTCGACTTAATCAGTAAACTATC AGCGAAATATGAGAAAACGAAAACAGAGAATGCACTGAGGGTGCCTGCTATA TGCAGGGACCTCACGACACTTATCTAGTGCAGCCTCTACTACGAGAATAAGGTA TACTCGCCACGTTACGGCGGTTCTAAAGGCGTTATGAAGATTATGCACTCTG TCTGATTGTGAAAGGGAGCGGCTGATATTGCAAGACAATATAAAAGATAATGAG GTCATCAAGCAGAATGGAAGTCGTTACTACTCCTCATACGCTTACTCCAATATGT ACTCTCGCAGAAGCGACGTACGATTGATACCCCTACCGCATCGTCAGCGACGAGT ACAAGATGTACAATTCTGTTGTCTTCAGATGAGAAGTCTGCCGGAAAGGAG TTCAGCAGATTCTGAGAATTCAAGACTATCTGGGTTGAGCATCGCTGAGAA GTTATCCCAGAAGGAGTACAGCTCGGTACGGAGTACGAGCGATTGAAGGAGGTA CACGTGAAGTCTGTTAAACATTATTGTCAGATCCTCGCTCGTAGCGACGAGTCT GACATAAGTAAAGTGTATCTCACTGAGAAGGGCGTGGAGATGTTGGTAAGATACT ATACCAGAGACCTATTCTAAAGGAAACGAGAAGGCCAACGCCAACGCGGTCAATG

	AGTCATATCTCCTCCTATTCAAGGTGAAGTACTACTTTAACAAATTGGAAAGGACG GTGTTATCCTGAGCCCAAGCGACAGCTTGAGAAGAAATGAGAACACTTATGTTGAA GGAGCAGAAGCATATAACAGAGAAGTTGAAATGtaa
NLS	CCCAAGAAAAAGCGCAAGGTA or CCAAAAAAGAAAAGAAAAGTT or CCCAAGAAAAAGAGAGAAAGGTG (amino acid sequence: PKKKRKV)
HAtag	TACCCATACGACGTCCCAGACTACGCT or TATCCTTATGATGTTCTGATTATGCA
HIStag	CATCATCATCATCATCAC
GS linker	GGAAGT or GGATCC or GGCTCC (amino acid sequence: GS)
CYC1t_atc	CATGTAATTAGTTATGTCACGCCCTACATTACGCCCTCCCCCACATCCGCTCTAACCG AAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTATTTTTTTAGTTAT GTTAGTATTAAGAACGTTATTATTTCAAATTTCCTTTTTCTGTACAGACGCG TGTACGCATGTAACATTACTGAAAACCTTGCTTGAGAAGGTTTGGGACGCTCGA AGGCTTAATTGCAAGCTatc
Tsynth24	TGGGTGGTATGTTATATAACTGTCTAGAAATAAGAGATCATCTTCAAA
ADH1t	GCGAATTCTTATGATTATGATTATTATTAAATAAGTTAAAAAAAATAAGT GTATACAAATTAAAGTGACTCTTAGGTTAAACGAAAATTCTTATTCTGAGT AACTCTTCTGTAGGTCAGGTTGCTTCTCAGGTATAGCATGAGGTCGCTTTAT TGACCACACCTCTACCGGCATG
SUP4t	TTTTTTGTTTTATGTCT
donor DNA 1 (Combines with sgRNA1, sgRNA2, and sgRNA3)	ttccttggccaacttggttacgacttgtctcacgggttcaatgttcGGGCTGATCATTAACTATC CACTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCCAGC ACTCTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTATTTCTCCATGA AGATGGTACAAGACTGGGTGAGCATCTGGTGCATTGGGACACAGGaaagatg gtaacatttgggtcacaagttagaatacaattcaact
donor DNA 2 (Combines with sgRNA4, sgRNAs5, and sgRNA6)	atatttacattatggctttaagcaaagaacggattaaaggtaacttGGGCTGATCATTAACTAT CCACTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCCAG CACTCTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTATTTCTCCATG AAGATGGTACAAGACTGagagatcacatggctttagaatttcgtactgctgctggatcactt

Table S3. sgRNA sequences used in this work.

NAME	LOCATION	SPACER SEQUENCE	DIRECT REPEAT	FIGURE
sgRNA1_SpyCas9	250...269	TCATGTTGCTTCATGTGATC	GTTTAGAGCT AGAAATAGCAA GTTAAAATAAG GCTAGTCCGTT ATCAACTTGAA AAAGTGGCACCC GAGTCGGTGGT GC	1C, 2C
sgRNA2_SpyCas9	284...303	AACGTAACCTTCTGGCATGG		
sgRNA3_SpyCas9	381...400	AGTCAATAGAACATGAATTGA		
sgRNA4_SpyCas9	523...542	ATCAGACACAACGTTGAAGA		
sgRNA5_SpyCas9	601...620	TGAGAACATCTGGTAATAAGAC		
sgRNA6_SpyCas9	636...655	GGTCCTTAGACAAGACAGAT		
non-target sgRNA_SpyCas9	–	AAGTCTTCGTCTGAAGACAA		
sgRNA1_Nme1Cas9	240...263	CCGTTACCCAGATCACATGAA GCA	GTTTAGCTCC CTTTCTCATTT GGAAACGAAAT GAGAACCGTTG CTACAATAAGG CCGCTCTGAAAA GATGTGCCGCA ACGCTCTGCC CTTAAAGCTTCT GCTTTAAGGGG CATCGTTA	3C
sgRNA2_Nme1Cas9	286...309	TTCTTGACGTAACCTTCTGGC AT		
sgRNA3_Nme1Cas9	381...404	AGTCAATAGAACATGAATTGAA GGG		
sgRNA4_Nme1Cas9	530...553	GGACAGAACCGTCTTCAACGT TGT		
sgRNA5_Nme1Cas9	585...608	AATTGGTGACGGTCCAGTCTT ATT		
sgRNA6_Nme1Cas9	643...666	CTTTTCATTGGGTCTTAGAC AA		
non-target sgRNA_Nme1Cas9	–	ACCAGGCCAGCCATGTTCTG CCA		
sgRNAr1_Nme1Cas9	104...127	AATTGACCAAAATAGGGACA ACA	GTTTAGCTCC CTTTCTCATTT GGAAACGAAAT GAGAACCGTTG CTACAATAAGG CCGCTCTGAAAA GATGTGCCGCA ACGCTCTGCC CTTAAAGCTTCT GCTTTAAGGGG CATCGTTA	3C
sgRNAr2_Nme1Cas9	134...157	GAGAACTTGTGACCGTTGACA TCA		
sgRNAr3_Nme1Cas9	194...217	CAGATGAACTCAAGGTCAAC TTA		
sgRNAr4_Nme1Cas9	223...246	GCCAAGGAAACAGGCAACTTA CCAG		
sgRNAr5_Nme1Cas9	315...338	CATGGCGGATTAAAGAAATC ATG		
sgRNAr6_Nme1Cas9	347...370	AAGATAGTTCTTCTTGAACGT AA		

sgRNAr7_Nme1Cas9	406...429	AAGTATCACCTTCGAACCTGAC CTT		
sgRNAr8_Nme1Cas9	455...478	TTACCATCTCCTTGAAATCAA CA		
sgRNAr9_Nme1Cas9	488...511	TTGAAATTGTATTCTAACTTGT GA		
sgRNAr10_Nme1Cas9	554...577	CTGATTTAAAGTTGACCTTA TA		
sgRNAr11_Nme1Cas9	596...619	TAGTGGTCAGCTAATTGGACA GAA		
sgRNAr12_Nme1Cas9	624...647	ACCGTCACCAATTGGAGTGTT TTG		
sgRNAr13_Nme1Cas9	730...753	CAGCAGTACGAAATTCTAAC AGA		
sgRNAr14_Nme1Cas9	812...835	GATGATACTCTTATTCTAGA CA		
sgRNAr1_LwaCas13a	21...48	TGTTTGTGTCTATAGAAGTAG TAGTAA	GATTAGACTAC CCCAAAAACGA AGGGGACTAAA AC	4B, S2, S3B
sgRNAr2_LwaCas13a	76...103	ACACCAGTGAATAATTCTTCAC CTTTAG		
sgRNAr3_LwaCas13a	331...358	CTTTCTGAACATAACCTTCTG GCATGG		
sgRNAr4_LwaCas13a	591...618	GATAATGGTCAGCTAATTGAA CAGAAC		
sgRNAr1_RfxCas13d	21...42	TGTGTCTATAGAAGTATAGTAA	CACTAGTGC GATTGC ACTAGT CTAAAAC (named DR_UrCas13d)	4B, S2, S3B
sgRNAr2_RfxCas13d	76...97	GTGAATAATTCTCACCTTAC		
sgRNAr3_RfxCas13d	331...352	TGAACATAACCTCTGGCATG G		
sgRNAr4_RfxCas13d	591...612	GGTCAGCTAATTGAACAGAAC C		
pre- crRNA_LwaCas13a (The underlined sequences in italics are the spacers; the other sequences are the direct repeats)	79...106 116...143 265...292 79...106 116...143 265...292	GATTAGACTACCCCCAAAAACGAAGGGGACTAAA <u>ACACAA</u> CAC <u>CCAGTGA</u> ATAATT <u>CTTCACCTT</u> GATT GA <u>CTACCC</u> AAAAACGAAGGGGACTAAA <u>ACTATCTGCAAA</u> <u>ACATTGA</u> AC <u>ACCCATAACG</u> ATT <u>AGACTACCC</u> AAA AACGAAGGGGACTAAA <u>ACAA</u> AC <u>ACCCAGTGA</u> ATA <u>ATTCTCACCTT</u> GATT <u>AGACTACCC</u> AAA <u>ACGA</u> AGGGGACTAAA <u>ACCCATTAAAC</u> <u>ATCACCATCTA</u> ATT <u>CAACCG</u> ATT <u>AGACTACCC</u> AAA <u>ACGA</u> AGGGG CTAAA <u>ACTATCTGCAAA</u> ACATT <u>GAACACCATAAC</u>		

sgRNAr1_RfxCas13d	21...48	TGTTTGTGTCTATAGAAGTA TAGTAA	<u>RfxCas13d(stan</u> <u>dard DR):</u> AACCCCTACCA ACTGGTCGGGG TTGAAAC	S3A
sgRNAr2_RfxCas13d	76...103	ACACCAGTGAATAATTCTCAC CTTTAG	<u>RfxCas13d(opti</u> <u>mal DR):</u> TACCCCTACCAA CTGGTCGGGGT TTGAAAC	
sgRNAr3_RfxCas13d	331...358	CTTTCTTGAACATAACCTTCTG GCATGG		S3C

*The location of each sgRNA represents the target sequence (on either *yEBFP2* or *yEGFP* gene) with respect to the transcription start site of corresponding promoter.

Table S4. Yeast strains engineered in this work.

NAME	GENOTYPE
bYMM584	CEN.PK2-1C (MATa; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2)
bYMM1370	bYMM584 pMM1309::TRP1
bYMM1379	bYMM584 pMM1309::TRP1 pMM1303::URA3
bYMM1380	bYMM584 pMM1309::TRP1 pMM1321::URA3
bYMM1381	bYMM584 pMM1309::TRP1 pMM1322::URA3
bYMM1386	bYMM584 pMM1309::TRP1 pMM1303::URA3 pMM1328::LEU2
bYMM1387	bYMM584 pMM1309::TRP1 pMM1303::URA3 pMM1329::LEU2
bYMM1388	bYMM584 pMM1309::TRP1 pMM1303::URA3 pMM1330::LEU2
bYMM1389	bYMM584 pMM1309::TRP1 pMM1322::URA3 pMM1325::LEU2
bYMM1390	bYMM584 pMM1309::TRP1 pMM1322::URA3 pMM1326::LEU2
bYMM1391	bYMM584 pMM1309::TRP1 pMM1322::URA3 pMM1327::LEU2
bYMM1409	bYMM584 pMM1309::TRP1 pMM1303::URA3 pMM1352::LEU2
bYMM1410	bYMM584 pMM1309::TRP1 pMM1303::URA3 pMM1353::LEU2
bYMM1411	bYMM584 pMM1309::TRP1 pMM1303::URA3 pMM1354::LEU2
bYMM1412	bYMM584 pMM1309::TRP1 pMM1322::URA3 pMM1349::LEU2
bYMM1413	bYMM584 pMM1309::TRP1 pMM1322::URA3 pMM1350::LEU2
bYMM1414	bYMM584 pMM1309::TRP1 pMM1322::URA3 pMM1351::LEU2
bYMM1415	bYMM584 pMM1309::TRP1 pMM1321::URA3 pMM1349::LEU2
bYMM1416	bYMM584 pMM1309::TRP1 pMM1321::URA3 pMM1350::LEU2
bYMM1417	bYMM584 pMM1309::TRP1 pMM1321::URA3 pMM1351::LEU2
bYMM1436	bYMM584 pMM1309::TRP1 pMM1303::URA3 pMM1402::LEU2
bYMM1437	bYMM584 pMM1309::TRP1 pMM1322::URA3 pMM1381::LEU2
bYMM1418	bYMM584 pMM1379::TRP1
bYMM1424	bYMM584 pMM1379::TRP1 pMM1321::URA3
bYMM1456	bYMM584 pMM1379::TRP1 pMM1424::URA3
bYMM1458	bYMM584 pMM1379::TRP1 pMM1424::URA3 pMM1426::LEU2
bYMM1459	bYMM584 pMM1379::TRP1 pMM1424::URA3 pMM1427::LEU2
bYMM1460	bYMM584 pMM1379::TRP1 pMM1424::URA3 pMM1428::LEU2
bYMM1461	bYMM584 pMM1379::TRP1 pMM1424::URA3 pMM1429::LEU2
bYMM1462	bYMM584 pMM1379::TRP1 pMM1424::URA3 pMM1430::LEU2
bYMM1463	bYMM584 pMM1379::TRP1 pMM1424::URA3 pMM1431::LEU2

bYMM1464	bYMM584 pMM1379::TRP1 pMM1424::URA3 pMM1445::LEU2
bYMM1465	bYMM584 pMM1379::TRP1 pMM1424::URA3 pMM1453::LEU2
bYMM1466	bYMM584 pMM1379::TRP1 pMM1424::URA3 pMM1446::LEU2
bYMM1467	bYMM584 pMM1379::TRP1 pMM1424::URA3 pMM1447::LEU2
bYMM1468	bYMM584 pMM1379::TRP1 pMM1424::URA3 pMM1448::LEU2
bYMM1469	bYMM584 pMM1379::TRP1 pMM1424::URA3 pMM1454::LEU2
bYMM1470	bYMM584 pMM1379::TRP1 pMM1424::URA3 pMM1455::LEU2
bYMM1471	bYMM584 pMM1379::TRP1 pMM1424::URA3 pMM1380::LEU2
bYMM600	bYMM584 pMM728::LEU2
bYMM666	bYMM584 pMM728::LEU2 pMM813::URA3
bYMM737	bYMM584 pMM728::LEU2 pMM843::URA3
bYMM844	bYMM584 pMM728::LEU2 pMM813::URA3 pMM805::TRP1
bYMM845	bYMM584 pMM728::LEU2 pMM813::URA3 pMM806::TRP1
bYMM846	bYMM584 pMM728::LEU2 pMM813::URA3 pMM792::TRP1
bYMM847	bYMM584 pMM728::LEU2 pMM813::URA3 pMM793::TRP1
bYMM848	bYMM584 pMM728::LEU2 pMM843::URA3 pMM839::TRP1
bYMM849	bYMM584 pMM728::LEU2 pMM843::URA3 pMM840::TRP1
bYMM850	bYMM584 pMM728::LEU2 pMM843::URA3 pMM841::TRP1
bYMM851	bYMM584 pMM728::LEU2 pMM843::URA3 pMM842::TRP1
bYMM852	bYMM584 pMM728::LEU2 pMM813::URA3 pMM888::TRP1
bYMM853	bYMM584 pMM728::LEU2 pMM813::URA3 pMM889::TRP1
bYMM854	bYMM584 pMM728::LEU2 pMM843::URA3 pMM890::TRP1
bYMM855	bYMM584 pMM728::LEU2 pMM843::URA3 pMM891::TRP1
bYMM856	bYMM584 pMM728::LEU2 pMM813::URA3 pMM933::TRP1
bYMM857	bYMM584 pMM728::LEU2 pMM813::URA3 pMM934::TRP1
bYMM858	bYMM584 pMM728::LEU2 pMM843::URA3 pMM935::TRP1
bYMM859	bYMM584 pMM728::LEU2 pMM843::URA3 pMM936::TRP1
bYMM860	bYMM584 pMM728::LEU2 pMM813::URA3 pMM673::TRP1
bYMM957	bYMM584 pMM728::LEU2 pMM843::URA3 pMM995::TRP1
bYMM959	bYMM584 pMM728::LEU2 pMM843::URA3 pMM997::TRP1
bYMM1005	bYMM584 pMM728::LEU2 pMM843::URA3 pMM1062::TRP1
bYMM1006	bYMM584 pMM728::LEU2 pMM843::URA3 pMM1063::TRP1
bYMM1007	bYMM584 pMM728::LEU2 pMM843::URA3 pMM1064::TRP1
bYMM1008	bYMM584 pMM728::LEU2 pMM843::URA3 pMM1065::TRP1

bYMM1010	bYMM584 pMM728::LEU2 pMM843::URA3 pMM995::TRP1 pMM1022::HIS3
bYMM1011	bYMM584 pMM728::LEU2 pMM843::URA3 pMM995::TRP1 pMM1023::HIS3
bYMM1013	bYMM584 pMM728::LEU2 pMM843::URA3 pMM997::TRP1 pMM1022::HIS3
bYMM1014	bYMM584 pMM728::LEU2 pMM843::URA3 pMM997::TRP1 pMM1023::HIS3
bYMM993	bYMM584 pMM728::LEU2 pMM843::URA3 pMM839::TRP1 pMM1022::HIS3
bYMM994	bYMM584 pMM728::LEU2 pMM843::URA3 pMM840::TRP1 pMM1022::HIS3
bYMM995	bYMM584 pMM728::LEU2 pMM843::URA3 pMM890::TRP1 pMM1022::HIS3
bYMM996	bYMM584 pMM728::LEU2 pMM843::URA3 pMM935::TRP1 pMM1022::HIS3
bYMM997	bYMM584 pMM728::LEU2 pMM843::URA3 pMM839::TRP1 pMM1023::HIS3
bYMM998	bYMM584 pMM728::LEU2 pMM843::URA3 pMM840::TRP1 pMM1023::HIS3
bYMM999	bYMM584 pMM728::LEU2 pMM843::URA3 pMM890::TRP1 pMM1023::HIS3
bYMM1000	bYMM584 pMM728::LEU2 pMM843::URA3 pMM935::TRP1 pMM1023::HIS3